



Genetic and Epigenetic Networks
in Cognitive Dysfunction

ENCODEY

Conference

2nd International



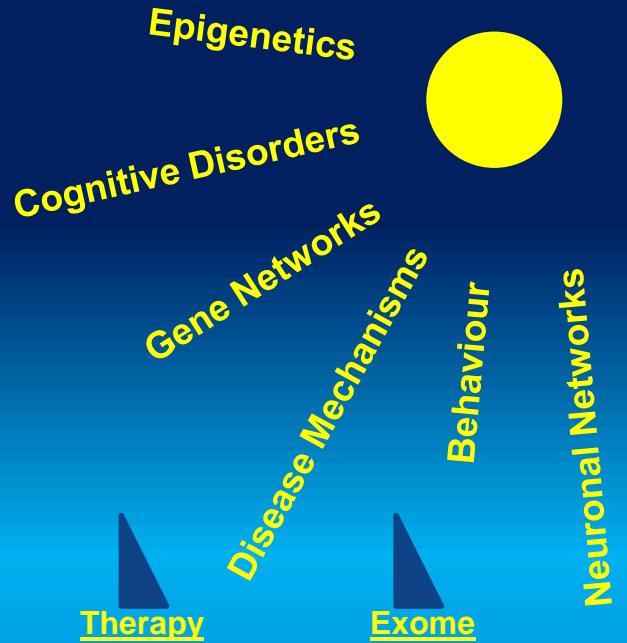
Integrative Networks in Intellectual Disabilities

27-29 April, 2015

Panorama Hotel

Chania, Crete

Greece



Conference book



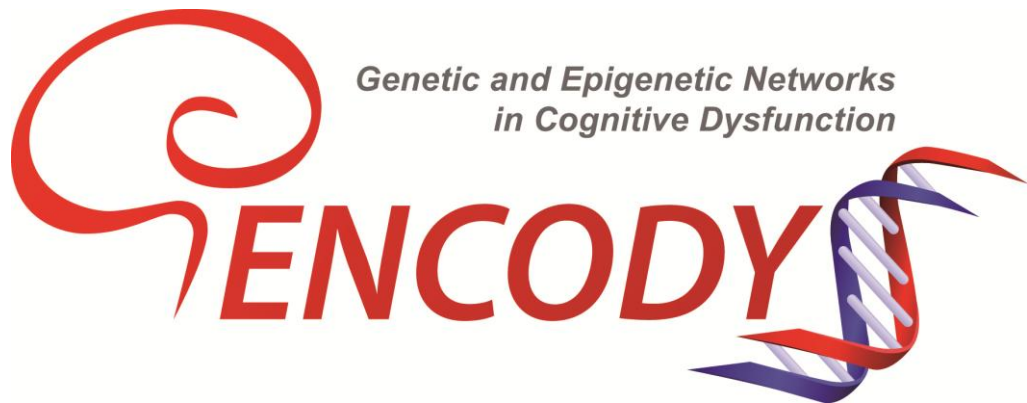
2nd INTERNATIONAL GENCODYS CONFERENCE

“Integrative Networks in Intellectual Disabilities”

27-29 April, 2015

**Panorama Hotel
Chania, Crete, Greece**

MAIN SPONSOR AND ORGANISERS:



**EU project: GENCODYS
Genetic and Epigenetic Networks in Cognitive Dysfunction**

**Large scale functional genomics effort in multi-cellular organisms to elucidate
the function of human gene products**

**EU 7th Framework Program Grant Agreement no.: 241995
www.Gencodys.eu**

WELCOME

Dear participant,

A very warm welcome at the second International Gencodys Conference. We hope that you will enjoy the scientific contents of this conference as well as its lovely settings, provided by the beautiful island of Crete and the hospitality of its people!

The increasing power of sequencing allows the elucidation of causative genetic defects and risk factors in cognitive disorders (CD) by analysis of entire exomes and even complete genomes. A wide variety of chromosomal aberrations and a bewildering number of single gene mutations underlie intellectual disability (ID), and in a growing number of examples share a common etiology with other cognitive defects such as autism spectrum disorders and schizophrenia. Elucidation of the complete landscape of all CD-associated genes will allow us to recognize the underlying common pathological mechanisms. Already now, extensive functional interactions are seen between ID-associated proteins and intricate networks are becoming apparent. Examples include protein networks driving synaptic morphology and plasticity and the epigenetic orchestration of neuronal gene expression.

The European funded research consortium GENCODYS exploits a multilevel approach to resolve the integrative networks in intellectual disabilities. We are bringing together top researchers with complementary expertise and patient representatives to apply a systems biology approach to reveal the common molecular and cellular mechanisms leading to cognitive impairment and translational research possibilities. Our overall concept that also will be strongly reflected in the program of our conference is to: (1) Identify novel genes involved in cognitive disorders; (2) Elucidate associated molecular networks that are commonly disrupted in CD; and (3) Develop strategies that are capable to modulate the disease phenotype.

Thanks to you we were able to bring together top researchers, medical doctors and patient representatives, who represent the top in Cognitive Research and related activities. All presentations are related to studies of cognitive dysfunction but in widely varying fields, including genetics, cellular, molecular and physiological studies, (epi)genomics and bioinformatics. Integrative network approaches and focus on overlapping disease mechanisms between different disorders are prioritized. Importantly we have a session titled "improving patient care" in which the challenges and opportunities for translational research and how the target community can help to guide pre-clinical research to tailor their needs is addressed. Our joined effort and interaction during this meeting will help us broaden our insights in and understanding of Intellectual Disabilities and generate chances to build solid collaborations.

We are glad you chose to participate and share the exciting developments in neurogenetics-driven cognitive research! The future looks bright for Integrative Networks in Cognitive Dysfunction!

Hans van Bokhoven, on behalf of the organizing committee

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GENERAL INFORMATION

Scientific Committee:

- Prof. Hilger Ropers
- Dr. Annette Schenck
- Prof. Yann Herault
- Prof. Seth Grant
- Prof. Martijn Huynen
- Dr. Frédéric Laumonnier
- Prof. Hans van Bokhoven
- Dr. Dik Hagenbeek

Organizing Committee:

- Prof. Hans van Bokhoven
- Dr. Dik Hagenbeek

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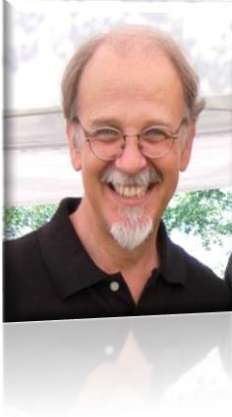
Scientific Program

Day/Time	Session/ Presentation Title	Speakers
Sunday 26 April 2015		
Evening	19:00-21:00	Welcome reception with finger foods and drinks.
Monday 27 April 2015		
Morning	Session 1	Genes and genetic networks disrupted in Intellectual Disability
09:00-09:30	- Genetic & Epigenetic Pathways of Disease	- Hans van Bokhoven
09:30-10:00	- Intellectual disability and related disorders: genetic progress and remaining challenges	- Hans-Hilger Ropers
10:00-10:15	- Pathway analyses of whole genome sequence data identifies novel candidate Intellectual Disability genes	- Farah Zahir
10:15-10:30	- Genetic and molecular basis of ID in Pakistani populations	- Sheikh Riazuddin
10:30-11:00	Coffee break	
	Session 2	Building bridges across Cognitive Disorders (CD)
11:00-11:30	- Transcriptomes, twins, and single cells: delightful liaisons	- Stylianos Antonarakis
11:30-12:00	- Developmental disorders, genetic interactions and a functionally-clustered genome	- Caleb Webber
12:00-12:15	- GABA/Glutamate synaptic pathways targeted by integrative genomic and electrophysiological explorations distinguish autism from intellectual disability	- Frédéric Laumonier
12:15-12:30	- A miRNA signature emphasizes epigenetic misregulation in Autism Spectrum disorders	- Lam Son Nguyen
12:30-14:00	Lunch break	
	Session 3	Epigenetic mechanisms in CD
14:00-14:30	- Gene regulation dynamics and chromatin architecture during development and evolution	- José-Luis Gómez-Skarmeta
14:30-15:00	- Reading the code: Epigenetic mechanisms in brain diseases	- André Fischer
15:00-15:15	- EHMT1/2 mediated histone methylation underlies homeostatic synaptic scaling by targeting BDNF	- Marco Benevento
15:15-15:30	- The role of EHMT1 and MLL3 in learning and memory	- Tom Koemans
15:30-16:00	Coffee break	
Afternoon	Session 4	Disease mechanisms in CD
16:00-16:30	- From molecules to behaviour: disentangling FXS and ASD	- Claudia Bagni
16:30-17:00	- High throughput standardized investigation of mouse	- Yann Hérault

	models in Cognitive Dysfunctions: The GENCODYS experience.	
17:00-17:15	- Novel mutations in <i>IL1RAPL1</i> associated with intellectual disability impair synapse formation	- Pierre Billuart
17:15-17:30	- E3 ubiquitin ligase <i>RLIM/RNF12</i> defects lead to a novel X-linked intellectual disability disorder in which the cognitive/behavioral phenotype of carrier females is rescued by favorable nonrandom X-inactivation	- Suzanna Frints
Evening	18:00	Poster Session with drinks
	20:00	Dinner
Tuesday 28 April 2015		
Morning	Session 5	Molecular mechanisms in CD: how mutations disrupt synaptic protein machines
09:00-09:30	- How is our behavioural repertoire built?	- Seth Grant
09:30-10:00	- Highly translational touchscreen phenotyping of mice bearing disease-relevant mutations	- Alexa Horner
10:00-10:15	- GABAergic synaptic plasticity in medial prefrontal cortex of <i>Fmr1</i> -KO mouse model: timing and time windows	- Rhiannon Meredith
10:15-10:30	- <i>NONO</i> mutations cause syndromic intellectual disability and inhibitory synaptic defects	- Laurence Colleaux
10:30-11:00	Coffee break	
	Session 6	Molecular and cellular mechanisms of CD
11:00-11:30	- Insights from Genomic approach into the understanding of human brain development	- Jamel Chelly
11:30-12:00	- Mitochondrial Dysfunction in Intellectual Disability	- Patrik Verstreken
12:00-12:15	- Phenotypic variability between <i>OCRL</i> -mutated fibroblasts from patients with Dent-2 disease or Lowe syndrome	- Olivier Dorseuil
12:15-12:30	- Clinical, genomic and functional characterization of 2p15.3-16.1 microdeletion syndrome	- Evica Rajcan-Separovic
12:30-12:45	- Characterization of the <i>Arx</i> c.428_451dup24 KI mouse line, model of ARX most frequent mutation	- Aline Dubos
12:45-14:15	Lunch break	
Afternoon	15:00	Social Event: Visit Chania
	20:00	Dinner and Party
Wednesday 29 April 2015		
Morning	Session 7	Genomics in cognition across species
09:00-09:30	- Identifying the molecular systems disrupted in ID and their genes	- Martijn Huijnen
09:30-10:00	- Human Phenotype Ontology: Algorithms and Applications	- Peter Robinson

10:00-10:15	- 9.6% of mouse gene knockouts show abnormal neuroanatomy: a resource to identify genes related to intellectual disability in human	- Binnaz Yalcin
10:15-10:30	- Using high-throughput light-off jump reflex habituation to understand learning deficits in <i>Drosophila</i> models of ID	- Michaela Fenckova
10:30-11:00	Coffee break	
	Session 8 Pre-clinical studies towards therapeutic intervention	
11:00-11:30	- Developing a fruit fly neuro-behaviour test battery	- Zoltan Asztalos
11:30-12:00	- Mouse models for rare disorders: from mechanisms to trials	- Ype Elgersma
12:00-12:15	- Investigating <i>Dyrk1a</i> gene dosage effect in glutamatergic neurons in a mouse model for Down syndrome	- Véronique Brault
12:15-12:30	- Haploinsufficiency of MECP2-interacting transcriptional co-repressor SIN3A causes mild intellectual disability by affecting the development of cortical integrity	- Tjitske Kleefstra
12:30-14:00	Lunch break	
Afternoon	Session 9 Diagnosis of CD: "common practice of prevention, present and future"	
14:00-14:30	- De novo mutations in intellectual disability	- Joris Veltman
14:30-15:00	- Whole Exome Sequencing in Research and Diagnosis of Intellectual Disability	- Hossein Najmabadi
15:00-15:15	- Exome sequencing in patients with Circumferential skin creases Kunze type: Evidence for locus heterogeneity	- Hilde Van Esch
15:15-15:30	- How to make patients benefit more from genetic research and genetic research from patients	- Cor Oosterwijk
15:30-16:00	Coffee break	
	Session 10 Strategies for therapeutic intervention: improving care for patients	
16:00-16:30	-Translating molecular advances into therapy	- Sébastien Jacquemont
16:30-17:00	-Rare Chromosome Disorder Support Group, 'Unique'	- Sarah Wynn
17:00-17:30	-Patient oriented planning for optimal translational strategy	- Florence Bietrix
Evening	20:00	Dinner

INVITED SPEAKERS



Stylianos E. Antonarakis is currently Professor and Chairman of Genetic Medicine at the University of Geneva Medical School, and the founding director of iGE3 (institute of Genetics and Genomics of Geneva). He is a medical, molecular, human geneticist, physician-scientist, who studied extensively the relationship between genomic and phenotypic variation. His research work includes the molecular bases of monogenic disorders and complex genetic disorders including the beta-thalassemias, hemophilias, and trisomy 21. His laboratory participated in the human genome sequence and functional analysis, particularly on chromosome 21. He is an international expert on disorders of chromosome 21, cloning of genes for genetic disorders, development of diagnostic tests, genome structure and function, studies of the genome variability, and conserved non-coding sequences in human DNA. He has published extensively (more than 620 well-cited papers) in the scientific literature, and is co-editor of the current edition of the classic textbook “Genetics in Medicine”; he is listed as one of the highly cited scientists by the ISI institute (more than 45,000 citations; h-index 104). He was the President of the European Society of Human Genetics (2001-2002), and President of HUGO for 2013-2016, foreign member of the Academy of Athens (2003), member of EMBO (2006). He was the co-organizer of the European School of Genetic Medicine, and in the last 28 years taught in the Bar Harbor Genetics Course, Maine. He was awarded the Society of Pediatric Research Young Investigator Award (1984), International Jerome Lejeune Prize (2004), the European Society of Human Genetics Award (2005), and was elected to the Society of Scholars of the Johns Hopkins University (2006), and the American Academy of Physicians (2010). He was awarded the Commander of the Order of Phoenix medal from the Hellenic Democracy (2007). More than 70 talented young scientists were trained in his laboratory (graduate students and postdoctoral fellows); in addition more than 25 young physicians were trained in the Medical Genetics Clinic of his department. With Haig Kazazian he has established one of the first molecular diagnostic laboratories in USA as early as 1982. He is a member of the Swiss National Science Foundation Research Council, and the Chair of the Genetics Review Panel of the EU ERC. His research laboratory was/is supported by grants from the National Institutes of Health, the European Union (including the European Research Council), and the Swiss National Science Foundation and numerous other Foundations including the Gebert and Lejeune Foundations. His is the originator of the World Down Syndrome Day (http://en.wikipedia.org/wiki/World_Down_Syndrome_Day). His current interests and research projects are the functional analysis of the genome, effect of human genetic variation to phenotypic variation, the molecular pathogenesis of trisomy 21 and polygenic phenotypes, the functional characterization of the conserved fraction of the genome, diagnostics and prevention of genetic disorders, and the societal implications of genetics and genome research. Recent key paper: **Domains of genome-wide gene expression dysregulation in Down's syndrome.** *Nature* 2014; 508(7496): 345-50

Title of presentation : **Transcriptomes, twins, and single cells: delightful liaisons**

Zoltan Asztalos is director of Aktogen Limited, Cambridge, UK. He has received his Ph.D. in biology at the Lorand Eotvos University, Budapest, Hungary. Before he founded Aktogen Ltd., a University of Cambridge start-up company, Zoltan studied the inheritance of innate and learned *Drosophila* behaviour as postdoctoral fellow in Cold Spring Harbor, Tokyo and Cambridge. Aktogen is set up to accelerate the discovery of drug targets and drugs to treat mental (Central Nervous System) disorders employing the fruit fly as model system. Recent Key Publication: **Automated measurement of *Drosophila* jump reflex habituation and its use for mutant screening.** J. Neurosci. Methods 2009; 182(1): 43-8



Title of presentation: **Developing a fruit fly neuro-behaviour test battery**



Claudia Bagni is professor at the Faculty of Medicine University of Rome and the KU Leuven and group leader at the Flemish Institute for Biotechnology. Her research focus is on cellular and molecular studies of synaptic plasticity and cancer in the context of intellectual disabilities. Memory formation and cognitive processes that rely on activity-dependent synaptic plasticity are affected by local protein synthesis and shaping of the synapses. Synaptic inputs dictate the time, place and amount of protein synthesis necessary for the single synapses. Dysregulation of these mechanisms leads to spine dysmorphogenesis and to a variety of neuropathological conditions including the most common form of inherited mental retardation, the Fragile X syndrome (FXS), which is due to the absence or mutation of a single protein, FMRP. FMRP is involved in multiple steps of neuronal messenger RNA metabolism. The work of her group, as well as the work of others, has shown that Autistic Spectrum Disorder (ASD), Schizophrenia (SCZ) as well as Alzheimer's Disease (AD) are linked to FMRP function. They aim at identifying molecular pathways that are impaired in FXS and other disabilities such as ASD and SCZ using mouse and fly models as well as cell lines from patients. One of the major goals is to understand the regulation of synaptic protein synthesis and actin remodeling during brain development in physiological and the above-mentioned pathological conditions. Recent key publication: **FMRP regulates multipolar to bipolar transition affecting neuronal migration and cortical circuitry.** Nat Neurosci. 2014 Dec; 17(12): 1693-700

Title of presentation: **From molecules to behaviour: disentangling FXS and ASD**

Hans van Bokhoven is full Professor of Molecular Neurogenetics at Radboud University Medical Center. His research, embedded at the intersection between the Departments of Human Genetics and the Department of Cognitive Neuroscience, studies the molecular underpinnings of development and functioning of the nervous system and its building blocks, the neurons and other cell types. The research is translational: from patient to the bench and back to the patient. The basis of his multidisciplinary research is genes that are underlying genetic disorders, such as intellectual disability, autism spectrum disorders, and neural migration disorders. Besides innovative genetics and genomics methodologies to identify causative gene mutations in neurodevelopmental disorders, his group applies a range of complementary *in vitro* and *in vivo* approaches to get insight into the mechanisms of disease and the normal physiological pathways that are regulated by these genes. Thus behavioral paradigms are studied in genetic animal models such as mouse and *Drosophila* and linked to neurobiological and neurophysiological pathways in primary neurons. In addition, patient-derived iNeurons derived via induced pluripotent stem cells are used. An important emphasis of his research is placed on the elucidation of epigenetic pathways interfering with learning and memory defects in intellectual disability and autism. Followed by his group's discovery that mutations in *EHMT1* give rise to Kleefstra syndrome, which is characterized with intellectual disability, ASD and facial characteristics as leading phenotypic features, the study of the epigenetic network around the *EHMT1* gene has become a leading topic of his research. It is his aim to understand how (haploinsufficient) mutations in this gene lead to neuropathology. In addition, he and his group have the ambition to use this knowledge to develop new strategies for intervention. Recent key publication: **The genetics of cognitive epigenetics**. *Neuropharmacology* 2014 May; 80: 83-94



Title of presentation: **Genetic & Epigenetic Pathways of Disease**



Jamel Chelly has degrees in medicine and human genetics. He was appointed as scientist by CNRS (Centre National de Recherche Scientifique, France). In 1991 he was awarded by the Cancer Research foundation (UK) a three year Post-doc fellow position and carried out his research at the Institute of Molecular Medicine in Oxford UK and contributed in the identification of several diseases-related genes. In 1995, he established at the Cochin Institute the Laboratory of Genetics and Pathophysiology of intellectual disability (ID) and neurodevelopmental disorders. He is a founding member of the European XLMR Consortium that has been instrumental in the remarkable progress in the field of ID and neuronal migration disorders. In September 2003, he was appointed as Professor at University Paris Descartes. Since September 2014, Professor Chelly moved to Strasbourg University - Medical School of Strasbourg, joined the IGBMC and established his research group "Genetics and Pathophysiology of neurodevelopmental and epileptogenic disorders". Objectives of his research programs, firmly anchored to genetic discoveries of his group, aim to better define and understand disrupted molecular, cellular and neurobiological processes underlying neuronal migration defects and malformations of cortical development (MCD), such as lissencephaly/pachygyria and polymicrogyria. Following

the identification of Doublecortin (DCX) gene and its implication in large spectrum of neuronal migration disorders (des Portes et al., Cell 1998), his group showed that doublecortin is a protein associated with microtubules (Francis et al., Neuron 1999) that stabilizes oligomers of tubulins (Moore et al., EMBO J. 2006). He recently showed that **mutations in TUBA1A** (collaboration with J Flint's group, Oxford University), **TUBB2B, TUBB3, TUBB5, TUBG1, FIF2A, KIF5C and DYNC1H1, are associated with MCD** (recent key publications, Poirier et al., 2013; Kielar et al., 2014). This confirms that microtubule-dependent mitotic and postmitotic processes are major players of cortical development and contributors to the pathogenesis of MCD.

Title of presentation: **Insights from Genomic approach into the understanding of human brain development**

Ype Elgersma is professor in the department of Neuroscience at the Erasmus University Medical Center and Scientific Director of ENCORE expertise Center. His laboratory seeks to get insight in the molecular and cellular basis of cognitive disability, and to use this knowledge to develop treatments. The laboratory is particularly interested in developmental disorders that are associated with intellectual disabilities, with a specific focus in disorders in the RAS-ERK and TSC-MTOR pathways and in Angelman Syndrome. Central to the approach is the use of genetically engineered mice. These mice are studied at the biochemical, cellular and behavioral level. In this way the lab hopes to understand the specific function of these genes and proteins in neuronal function, and to develop therapies. To translate the mouse findings to the clinic, Ype Elgersma was founder of the *ENCORE* expertise center for neuro-developmental disorders, which includes the national referral center for TSC, Angelman Syndrome and Neurofibromatosis. Several clinical trials are currently ongoing in this center. The *ENCORE* expertise center is an inter-departmental collaboration involving 9 departments, but with a particular strong participation of the departments of Pediatrics, Child Neurology, (Child) Psychiatry, Clinical Genetics and Neuroscience. Recent key publication: **TORC1-dependent epilepsy caused by acute biallelic Tsc1 deletion in adult mice**. Ann Neurol. 2013;74(4): 569-79



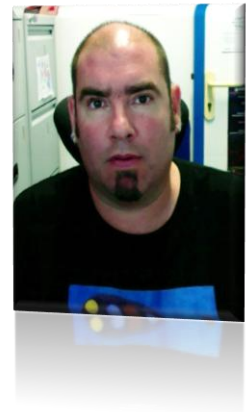
Title of presentation: **Mouse models for rare disorders: from mechanisms to trials**



Andre Fischer is professor for Epigenetic in Brain Diseases in the Department for Psychiatry and Psychotherapy, University Medical Center, Georg-August University Göttingen and speaker of the German Center for Neurodegenerative Diseases (DZNE) in Göttingen. His group investigates epigenetic mechanisms in neurodegenerative and neuropsychiatric diseases. To this end they pioneered next-generation sequencing approaches to study gene-expression networks in brain plasticity. They combine the analysis of human tissue with mechanistic studies in rodents employing behavioral and molecular approaches. Recent key publication: **K-Lysine acetyltransferase 2a regulates a hippocampal gene expression network linked to memory formation**. EMBO J. 2014;33(17): 1912-27

Title of presentation: **Reading the code: Epigenetic mechanisms in brain diseases**

José Luis Gómez Skarmeta is full professor at the Spanish National Research Council and Principal investigator at the Andalucian Centre for Developmental Biology in Seville, Spain. His areas of expertise are Developmental Biology (including *Drosophila*, *Xenopus* and zebrafish as animal models), Molecular Biology, Genetics, Functional Genomics and Epigenomics. In the last years he has been pioneering to combine recently developed molecular and developmental techniques to study the contribution of cis-regulatory elements and chromatin structure to development, evolution and human diseases. In 2009, he created the Aquatic Vertebrate Platform of CABD, an open research laboratory designed to facilitate the study of developmental mechanisms in lower vertebrates within a technological environment in continuous growth. This very successful Platform has been used by more than 50 researchers in the last 5 years from all around the world. Recent key publication: **Obesity-associated variants within FTO form long-range functional connections with IRX3**. Nature 2014 Mar 20; 507(7492): 371-75



Title of presentation: **Gene regulation dynamics and chromatin architecture during development and evolution**



Seth Grant graduated from Sydney University with a Bachelor of Science (Medicine) in Physiology, Bachelor of Medicine and Bachelor of Surgery. From 1985-1989 he was a Postdoctoral Fellow at Cold Spring Harbor Laboratory with Douglas Hanahan studying transgenic mouse models of cancer. From 1989-94 he studied mouse genetic models of learning and memory with Eric Kandel at Columbia University. He established his laboratory at the Centre for Genome Research at Edinburgh University in 1994 and in 2000 was appointed Professor of Molecular Neuroscience. In 2003 he was appointed Principal Investigator at the Wellcome Trust Sanger Institute in Cambridge and remained there until 2011, when he returned to Edinburgh University. He has held additional appointments including the John Cade Visiting Professor at Melbourne University, Honorary Professorship at Cambridge University and elected Fellow of the Royal Society of Edinburgh. His work focuses on the molecular basis of synapse function and behaviour. He

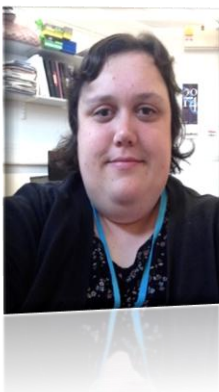
has characterized synaptic proteome organisation, evolution and function and identified the key role played by supramolecular assemblies of postsynaptic proteins. His synapse proteomic and genetic work has led to the identification of many diseases impacting on the synapse and the multiprotein complexes that control cognition.. Recent Key publication: **Synaptic scaffold evolution generated components of vertebrate cognitive complexity**. *Nat Neurosci*. 2013 Jan; 16(1): 16-24

Title of presentation: **How is our behavioural repertoire built?**

Yann Héroult is a Research Director at the CNRS, the French National Centre for Scientific Research, leading the “Institut Clinique de la Souris”, ICS (Mouse Clinical Institute, MCI-ICS, Illkirch), and a research group at the IGBMC (Illkirch). His main interest is oriented toward the identification of genes, sensitive to dosage, controlling the neurodevelopment and physiology. He focused on evaluating the consequences of gene dosage effect and copy number variation on cognition. He worked on Down Syndrome (DS, or Trisomy 21) and other intellectual disabilities (ID) attached to copy number variation. His objective is to identify candidate dosage sensitive genes, to further understand the pathophysiological mechanisms and to propose new therapeutic approaches to improve the deficit observed in patients. He developed several preclinical models of DS and other rare diseases causing ID. Using this panel of models he defined the contribution of several genomic regions to DS phenotypes affecting behavior and cognition, the cardiovascular system and the morphology. In addition he worked on DS candidate genes and developed a few therapeutic approaches to tackle down DS learning and memory phenotypes using specific drugs. Recent key publication: **The *App-Runx1* region is critical for birth defects and electrocardiographic dysfunctions observed in a Down syndrome mouse model**. *PLoS Genet*. (2012) 8, e1002724



Title of presentation: **High throughput standardized investigation of mouse models in Cognitive Dysfunctions: The GENCODYS experience**



Alexa Horner is the Behavioural Research Manager at Synome Ltd., a Cambridge-based UK biotechnology company. She is an expert in rodent touchscreen cognitive testing, which utilises a battery of highly standardised and translational tasks, many of which are analogous to those in the human Cambridge Neuropsychological Test Automated Battery (CANTAB). She heads a team of researchers who employ this technology platform to study the effects of mutations, natural genetic variation and drugs on the behaviour and cognition of mice. Recent key publication: **The touchscreen operant platform for testing learning and memory in rats and mice**. *Nat. Protoc*. 2013; 8(10): 1961-84

Title of presentation: **Highly translational touchscreen phenotyping of mice bearing disease-relevant mutations**

Martijn Huynen is a professor in comparative genomics at the Centre for Molecular and Biomolecular Informatics (CMBI), Radboud University Medical Centre.

He develops techniques to extract biomedically relevant information from genomics data to predict the functions of proteins and their interactions in pathways. Using these techniques he predicts new proteins of e.g. the mitochondrion and the cilium. The functions of multiple of his predicted proteins have been confirmed experimentally and the genes have been shown to be involved in intellectual disability. Recent key publication: **Mutations in the UQCC1-interacting protein, UQCC2, cause human complex III deficiency associated with perturbed cytochrome b protein expression.** PLoS Genet. 2013; 9(12): e1004034



Title of presentation: **Identifying the molecular systems disrupted in ID and their genes**



Sebastien Jacquemont is a professor of medical genetics at the University hospital of St. Justine and holds a Swiss national foundation assistant professorship as well as Canadian research chair.

SJ was trained as a clinical geneticist and subsequently completed a research fellowship in developmental pediatrics at the University of California, Davis where he developed expertise in fragile X syndrome (FXS). SJ was instrumental in characterizing a new FMR1-related neurological disorder for which he received in 2003 the clinical research award at the “Annual Meeting of the American Society of Human Genetics”. His strong interest in translational research has led him to work, in collaboration with Novartis Pharma, on developing and conducting clinical trials in patients with Fragile X syndrome. SJ

developed a second line of research investigating the impact of gene dosage on neurodevelopment, in particular neuropsychiatric and associated energy balance disorders. Recent key publication: **Investigation of memory, executive functions and anatomic, correlates in asymptomatic FMR1 premutation carriers.** Neurobiol Aging. 2014 Aug; 35(8): 1939-46

Title of presentation: **Translating molecular advances into therapy**

Hossein Najmabadi, PhD, professor of genetics, is the director and founder of the Genetics Research Center (GRC) at the University of Social Welfare and Rehabilitation Sciences in Tehran, Iran. The mandate of the GRC, also designated the National Reference Laboratory for Prenatal Diagnosis in Iran, is to prevent genetic disabilities and disorders by the establishment of a nationwide strategy for the early prenatal diagnosis of genetic disorders. In five areas of preventable genetic disorders, Dr. Najmabadi leads projects that not only apply preventive solutions within the population but also involve nationally and internationally collaborative research in order to improve the quality of life nationwide. The cognitive dysfunction in particular Intellectual Disability (ID) includes the evaluation of clinical heterogeneity of ID patients either syndromic or non-syndromic and the identification of genetic causes using



cytogenetics, molecular genetics techniques. He has identified many novel genes in Autosomal Recessive Intellectual Disability (ARID). In the study of both syndromic and non-syndromic deafness, he has identified the genes or mutations particular to Iran and established diagnostic protocols for them. In order to classify different subtypes of neuromuscular disorders (NMD) in Iran, family DNA studies are guided by the histopathology facilities at the GRC and applied Next Generation Sequencing (NGS). The investigation of hemoglobinopathies has also been conducted by him in a number of projects to identify the mutation spectrum of alpha- and beta-thalassemia, with the establishment of protocols for mutation identification and prenatal diagnosis. Moreover, studies on the potential elements in the induction of gamma globin as well as the molecular mechanism of hydroxyurea aim to improve the treatment of thalassemia. Recent key publication: **Deep sequencing reveals 50 novel genes for recessive cognitive disorders.** Nature 2011 Sep 21; 478(7367): 57-63

Title of presentation: **Whole Exome Sequencing in Research and Diagnosis of Intellectual Disability**



Peter Robinson, MD., PD, Msc., is Professor of Medical Genomics at the Charité University Hospital Berlin and Professor of Bioinformatics at the Free University Berlin. He has a BA. In Mathematics from Columbia College, an MD from the University of Pennsylvania, and a Master of Science in Computer Science from Columbia University School of Engineering. After an internship in primary care internal medicine at Yale, he completed a residency in Pediatrics at the Charité, and now leads a computational and wetlab research group at the Institute of Medical and Human Genetics of the Charité. The Robinson lab develops computational and experimental resources for the study of human biology and disease.

Recent highlights include the Human Phenotype Ontology (HPO), which is now an international standard for computation over human disease that is used by the Sanger Institute, several NIH-funded groups including the Undiagnosed Diseases Program, Genome Canada, the rare diseases section of the UK's 100,000 Genomes Project, and many others. We develop algorithms and software for the analysis of exome and genome sequences, including most recently the Exomiser, which was developed jointly with the Sanger Institute. The group has used whole-exome sequencing and other methods to identify a number of novel disease genes, including CA8, PIGV, PIGO, PGAP3, IL-21R, PIGT, and PGAP2. We support a range of genomics research projects at the Berlin Brandenburg Center for Regenerative Therapies (BCRT), including ChIP-seq, RNA-seq, T-cell receptor profiling, and deep-sequencing analysis of DNA methylation. We have identified secondary deleterious effects of a class of extracellular matrix fragment containing the motif Gly-x-x-Pro-Gly in Marfan syndrome, and have used this finding to develop a novel therapy in a mouse model of Marfan syndrome. Our focus in the coming decade will be on integrative computational analysis of genomics and clinical data in order to extend our understanding of human disease, personalized and systems medicine. Recent key publication: **Effective diagnosis of genetic disease by computational phenotype analysis of the disease-associated genome.** Sci Transl Med. 2014 Sep 3; 6(252): 252ra123.

Title of presentation: **Human Phenotype Ontology: Algorithms and Applications**

Hans-Hilger Ropers has recently retired as Director at the Max Planck Institute for Molecular Genetics (MPIMG) in Berlin and Professor for Human Genetics at the Free University Berlin (1994-2014), and as Chairman of the Biomedical Section of the (former Prussian) Berlin Brandenburg Academy of Science (BBAW, 2008-2014). From 1983 to 1997 he served as head of the Institute for Human Genetics at the University of Nijmegen, NL. As MD and first-generation Board-Certified Clinical Geneticist, he has a long-standing interest in the molecular elucidation, diagnosis, prevention and treatment of genetic disorders.



In the 1980ies, H.H. Ropers was an active member of the Gene Mapping Community, acting as Chromosome Chair and Co-Chair at all Human Genome Mapping Conferences between 1985 and 1993, and from 2003 to 2011, he served as Council and Program Committee member of the Human Genome Organization. In the early 1990ies, he and his coworkers were among the first to employ positional cloning strategies for systematically identifying the molecular causes of Mendelian disorders, with a focus on X-linked blindness, deafness and mental retardation. As member of the European X-linked Mental Retardation Consortium (*1995) they made seminal contributions to the elucidation and diagnosis of X-linked intellectual disability (XLID); together with their Danish partner, they were involved in the first systematic effort to characterize disease-associated balanced chromosome rearrangements; and they were also one of the first to describe copy number variants in complex disorders other than ID. In 2004, when XLID turned out to be less common than previously thought, H.H.Ropers and his Iranian partner set out to study autosomal recessive forms of ID (ARID) in a systematic manner. By pioneering the use of SNP arrays for large-scale autozygosity mapping in consanguineous ARID families they demonstrated that ARID is extremely heterogeneous. Another milestone was the early adoption of Next Generation Sequencing (NGS) technology in 2007 when the MPIMG became the first Continental European customer of Solexa (now Illumina). This added a new dimension to the molecular elucidation of X-linked and autosomal ID which is still being pursued by his group.

More recently, H. H. Ropers has been promoting the adoption of NGS techniques to improve genetic health care in Germany. After having analyzed his own genome, he believes that the time has come for introducing whole genome sequencing (WGS) as a first line diagnostic test for selected pediatric patients. At the same time, he is convinced that medical genome sequencing should be confined to experienced, suitably manned and equipped Competence Centers for Rare Diseases.

He is member of the Royal Dutch Academy of Sciences (since 2002) and the BBAW (since 2003), Honorary Member of the German Society of Human Genetics and recipient of its Medal of Honor (2009), and in 2014 he received the Scientific Award of the European Organization for Rare Diseases. Recent key publications: **De novo truncating mutations in ASXL3 are associated with a novel clinical phenotype with similarities to Bohring-Opitz syndrome.** *Genome Med.* 2013 Feb 5; 5(2): 11; **Integrated sequence analysis pipeline provides one-stop solution for identifying disease-causing mutations.** *Hum Mutat.* 2014 Dec; 35(12) 1427-35

Title of presentation: **Intellectual disability and related disorders: genetic progress and remaining challenges**



Joris A. Veltman is professor in Translational Genomics and head of the Genome Research division at the Department of Human Genetics, Radboud University Medical Centre in Nijmegen and the Department of Clinical Genetics, Maastricht University Medical Centre in Maastricht, The Netherlands.

His research focuses on the identification and interpretation of genomic variation, with a particular interest in the role of rare *de novo* mutations and copy number variations in severe neurodevelopmental and psychiatric diseases such as intellectual disability and rare genetic diseases. With his research group he studies the genomes of patients using next generation sequencing technology and combines laboratory experiments with novel bioinformatic approaches. In addition, he is actively involved in the

implementation of these novel genomics approaches in routine clinical diagnosis, aiming to improve the diagnostic yield, reduce the turn-around-time and make personalized medicine a reality. Recent key publication: **Genome sequencing identifies major causes of severe intellectual disability**. *Nature* 2014; 511(7509): 344-7

Title of presentation: **De novo mutations in intellectual disability**

Patrik Verstreken is professor at the KU Leuven and group leader at the VIB Center for the Biology of Disease in Belgium.

He uses fruit flies and mammalian cells to study neuronal and synaptic function and he creates and analyzes new disease models. His studies combine genetics with electrophysiology, imaging and electrophysiology. Recent key publication: **Mutations in the X-linked intellectual disability gene Ube2a cause neuronal dysfunction and impair Parkin-dependent mitophagy**. *Molecular Cell* 2013; 50: 831-43



Title of presentation: **Mitochondrial Dysfunction in Intellectual Disability**



Caleb Webber is programme leader in Neurological Disease Genomics at the Medical Research Council (MRC) Functional Genomics Unit at the University of Oxford. He obtained his PhD in 2003 from the European Bioinformatics Institute, The Wellcome Trust Genome Campus, Hinxton Cambridge and from the Department of Genetics, Cambridge University. Afterwards he returned to Oxford to work with Prof. Chris Ponting on most of the major large-scale genome projects of the last decade. Through CNVs, he became interested in the role of genetic variation in disease, applying novel functional genomics approaches to uncover the pathways and process disrupted in neurodevelopmental disorders, including intellectual disability, ADHD and autism. Over the last few years, his lab

has been drawn increasingly into elucidating the role of synergistic interactions in disease. Recent key publications: **The roles of FMRP-regulated genes in autism spectrum disorder: single- and multiple-hit genetic etiologies**. *AJHG* 2013; 93(5):825-39. **Unbiased functional clustering of gene variants with a phenotypic-linkage network**. *PLoS CompBio* 2014; 10(8):e1003815,

Title of presentation: **Developmental disorders, genetic interactions and a functionally-clustered genome**

PRESENTATION ABSTRACTS

1_Benevento

EHMT1/2 mediated histone methylation underlies homeostatic synaptic scaling by targeting BDNF

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Homeostatic plasticity is an important form of plasticity that is required to maintain a fine balance between overall excitation and inhibition in developing and mature neuronal networks. The best-characterized form of homeostatic plasticity is synaptic scaling expressed at glutamatergic synapses. Signalling pathways that sense enduring alterations in intracellular Ca^{2+} have been characterized to be involved in synaptic scaling through *de novo* gene transcription and epigenetic regulation. In particular the involvement of epigenetic factors in regulating synaptic scaling has recently gained more attention. However a precise model of the dynamic changes that involve epigenetic factors remains unknown.

Here we show that Euchromatic histone lysine methyl-transferase 1 (EHMT1) plays an essential and cell-autonomous role in synaptic scaling in response to reduced firing or reduced sensory drive in mouse visual cortex. Specifically, we found that in primary cortical neurons a chronic reduction in network activity, induced by TTX, increases H3K9me2 levels, the catalytic product of EHMT1. We show that genetic and pharmacological blockage of EHMT1 fails to increase H3K9me2 levels as well as miniature excitatory post-synaptic amplitudes. Similarly, we found that in EHMT1^{+/-} mice homeostatic synaptic scaling up in response to visual deprivation *in vivo* was impeded. Moreover, we demonstrate that *Ehmt1* down regulates BDNF expression during scaling up by increasing the deposition of H3K9me2 at the BDNF promoter. Together our data show that *Ehmt1* is a cell-autonomous epigenetic regulator that governs a repressive program required for synaptic scaling up.

2_Billuart

Novel mutations in *IL1RAPL1* associated with intellectual disability impair synapse formation

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Mutations in interleukin-1 receptor accessory protein like 1 (*IL1RAPL1*) gene have been associated with non-syndromic intellectual disability (ID) and autism spectrum disorder. *Il1rapl1* knock-out mice show altered learning and memory, reduction of dendritic spine density in hippocampus, and a deficit in long-term plasticity, suggesting a role of IL1RAPL1 in synapse formation, signaling and function. IL1RAPL1 is a member of interleukin 1 receptor family and it is located in excitatory synapses where it interacts with different partners, among them the scaffolding protein PSD-95 and the tyrosine phosphatase PTP δ , regulating the formation and function of excitatory synapses. Most of the reported mutations in human include multiple exons deletions and nonsense mutations.

The aim of the presentes work is to characterize the synaptic consequences of two novel *IL1RAPL1* mutations causing the deletion of exon 6 (Δ ex6) and one point mutation (C31R), identified in intellectual disability patients.

3_Braut

Investigating *Dyrk1a* gene dosage effect in glutamatergic neurons in a mouse model for Down syndrome

Véronique Braut, Javier Flores-Gutiérrez, Aline Dubos, Marie-Christine Birling, Guillaume Pavlovic, Mohammed Selloum, Tania Sorg, Valérie Lalanne, Hamid Méziane and Yann Hérault

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Cognitive impairment in Down syndrome (DS) has been linked to deficits in synaptic transmission and GABA-glutamate imbalance. The analysis of a mouse model of DS revealed an overproduction of the inhibitory neurotransmitter GABA that restricted synaptic activation of the glutamatergic receptors NMDA leading to LTP (Long Term Potentiation) and memory deficits. *DYRK1A* (Dual-specificity Tyrosine-(Y)-phosphorylation Regulated Kinase 1A) is a strong candidate for DS cognitive phenotypes. Transgenic mice overexpressing *Dyrk1a* have impairment in hippocampus-dependent memory and deficits in LTP. Pharmacological inhibition of DYRK1A by the green tea flavonol epigallocatechin-gallate (EGCG) in both trisomic and *Dyrk1a* transgenic mice rescued the memory deficit in those mice. Moreover, molecular analyses revealed imbalance of GABAergic and glutamatergic proteins in the brain of TgDyrk1a mice with the opposite phenotype observed in *Dyrk1a* knockout heterozygous mice. However, how *Dyrk1a* impacts the balance between the two pathways is still not clear. To decipher the role of Dyrk1a in the glutamatergic system, we used a conditional knockout allele of *Dyrk1a* with a transgenic mouse expressing the Cre recombinase under the CaMKII α promoter (Tg(CaMKII α -Cre)) expressed in hippocampal and cortical glutamatergic neurons. We mated those mice with the trisomic mouse model Ts1Yey to returning to two copies of *Dyrk1a* in glutamatergic neurons. We also produced *Dyrk1a*^{CaMKII α /+} and *Dyrk1a*^{CaMKII α /CamKII α} mice in order to look at the impact of the deficit of Dyrk1a in glutamatergic neurons. Returning to two copies of *Dyrk1a* in glutamatergic neurons was not sufficient to rescue working memory deficits observed in Ts1Yey mice, indicating that trisomy of *Dyrk1a* in glutamatergic neurons is not alone responsible for those deficits. However, object recognition was rescued in Ts1Yey/*Dyrk1a*^{CaMKII α /+} mice, indicating a major effect of *Dyrk1a* trisomy on the glutamatergic pathway in declarative memory. Removing one copy of *Dyrk1a* in glutamatergic neurons (*Dyrk1a*^{CaMKII α /+}) triggered object recognition memory deficit in the mice without altering working memory deficits. *Dyrk1a*^{CaMKII α /CamKII α} mice showed decreased hippocampus-dependent contextual freezing performance, decreased social memory performance, improved rotarod performance and increased exploration of the centre of the openfield suggesting decreased anxiety-related behavior. These findings correlate with drug normalization experiments and suggest that EGCG acts on glutamatergic neurons for rescuing object recognition.

4_Colleaux

NONO mutations cause syndromic intellectual disability and inhibitory synaptic defects

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Identifying causes of sporadic intellectual disability remains a considerable medical challenge. Here, we report on two unrelated patients presenting with intellectual disability, a slender build-macrocephaly, scoliosis, severe elocution disability with mandibulofacial dysostosis and a thick corpus callosum at brain MRI. High-throughput sequencing identified two distinct null mutations in the *NONO* X-linked gene: c.1131G>A, splice mutation, and c.1394dup; p.Asn466Lysfs*13.

NONO belongs to the highly conserved *Drosophila* Behaviour Human Splicing (DBHS) protein family. This family includes three members in mammals, namely *NONO*, paraspeckle component 1 (PSPC1), and Splicing Factor Proline/Glutamine-Rich (SFPQ, also known as PSF). DBHS proteins are nuclear proteins forming homo- and heterodimers *in vivo*, and previous literature documents their involvement in various aspects of RNA production. Studies *in vitro* suggest that they play a role in transcriptional activation and repression, splicing, pre-mRNA processing and RNA transport. In addition, they are major components of nuclear paraspeckles, which have been recognized as nuclear RNA-holding structures for edited RNAs that likely play a role in stress-mediated regulation via nuclear retention of transcripts. *NONO* and other DBHS family members also serve as transcriptional cofactors for correct circadian clock function in both flies and mammals, where they regulate the circadian clock via interaction with PER proteins. However, no study so far has linked impaired function of these proteins to human disease.

To further characterize the physiological role of *NONO* upon brain development, we analyzed a mouse model inactivated for the *Nono* gene. Comparing patients to *Nono*-deficient mice revealed related behavioral, craniofacial, and transcriptional anomalies. In brain, these mice also showed deregulation of a large number of synaptic transcripts including the GABA receptor alpha2 subunit, as well as impaired postsynaptic scaffolding of

gephyrin, a master organizer of inhibitory synapses. Importantly, alteration of synaptic scaffolding could be rescued by over-expression of Gabra2 in *NONO*-compromised neurons, suggesting that aspects of this syndrome are potentially treatable. Our data identify *NONO* as a new neurodevelopmental-disease gene and highlight the key role of DBHS proteins in functional organization of GABAergic synapses.

5_Dorseuil

Phenotypic variability between *OCRL*-mutated fibroblasts from patients with Dent-2 disease or Lowe syndrome.

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OCRL mutations are associated with both Lowe syndrome and Dent-2 disease, two rare X-linked conditions. Lowe syndrome is an oculo-cerebro-renal disorder, whereas Dent-2 patients mainly present renal proximal tubulopathy. Loss of OCRL-1, a phosphoinositide-5-phosphatase, leads in Lowe patients' fibroblasts to phosphatidylinositol-4,5-bisphosphate (PI(4,5)P2) accumulation, with defects in F-actin network, α -actinin distribution and ciliogenesis, whereas fibroblasts of Dent-2 patients are still uncharacterized.

We described for the first time that dermal Dent-2 fibroblasts with OCRL loss-of-function (LOF) mutations exhibit decrease in actin stress fibers, appearance of punctate α -actinin signals and alteration in primary cilia formation. Interestingly, we quantified these phenotypes as clearly intermediate between Lowe and control fibroblasts, thus suggesting that levels of these defects correlate with clinical variations observed between patients with OCRL mutations. In addition, we show that Lowe and Dent-2 fibroblasts display similar PI(4,5)P2 accumulation levels. Finally, we analyzed INPP5B, a paralogous gene already reported to exhibit functional redundancy with OCRL, and report neither differences in its expression, nor specific allelic variations between fibroblasts of patients.

Altogether, we describe here differential phenotypes between fibroblasts from Lowe and Dent-2 patients, both associated with OCRL LOF mutations, we exclude direct roles of PI(4,5)P2 and INPP5B in this phenotypic variability and we underline potential key alterations leading to ocular and neurological clinical features in Lowe syndrome.

6_Dubos

Characterization of the Arx c.428_451dup24 KI mouse line, model of ARX most frequent mutation

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The Aristaless related homeobox (ARX) is a transcription factor involved in the development of GABAergic and cholinergic neurons in the forebrain. The c.428_451dup24 duplication in the polyalanine tract 2 of ARX gene is the most frequent mutation identified and clinically gives rise to a spectrum of intellectual disabilities and seizures. In order to understand the physiological and functional consequences of this duplication, we generated and characterized a KI mouse model carrying the human c.428_451dup24 duplication (Arx dup24 KI).

The impact of the 24bp duplication was thoroughly analyzed in a variety of tests allowing evaluation of a wide range of CNS functions. Overall, Arx mutant males showed increased spontaneous activity (locomotion and rears) in different situations including the openfield, the Y-maze spontaneous alternation and the fear conditioning. Specific motor skills, shown to be altered in humans bearing the c.428_451dup24 mutation, were also affected in Arx dup24 KI mice, with altered grasping behaviour and altered gait. When analyzed for cognitive abilities, Arx mutants had altered contextual fear condition, while working memory and spatial learning were not affected in our experimental conditions.

We are currently analysing the consequence of the presence of the c.428_451dup24 duplication in the Arx gene on its transcriptional activity by a transcriptome analysis on forebrains from E15.5 embryos and on its role in GABAergic neurons migration by the study of the different interneurons subpopulation in the cortex, hippocampus and striatum of adult, P0 and E15.5 Arx WT and mutant males.

7_Fenckova

Using high-throughput light-off jump reflex habituation to understand learning deficits in *Drosophila* models of Intellectual Disability

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Impaired cognition is the central feature of Intellectual Disability (ID), a genetically and clinically highly heterogeneous group of disorders. Up to date, mutations in more than 650 genes are known to cause ID (“ID genes”). A number of them have already been shown to work in common molecular pathways, but paucity of functional data is hampering identification of such key cognition-regulating modules.

Our aim is to systematically describe the role of ID genes in neuronal function and cognition, define molecular modules through similar phenotypes and identify common targets for therapeutic intervention. To investigate the function of the large number of ID genes in learning, we use *Drosophila* as a model and a high-throughput light-off jump reflex habituation paradigm, where the startle jump response to repeated light-off stimuli gradually wanes. We have performed a systematic panneuronal RNA interference-mediated screen targeting *Drosophila* ID gene orthologues and identified a number of habituation mutants that are simultaneously being investigated for synaptic morphology. We would like to present the results of these efforts. Given that habituation is evolutionary conserved and results from a specific form of short-term synaptic plasticity, we aim to identify concrete synaptic pathways and processes that are disturbed in ID and lead to impaired habituation in ID *Drosophila* models. To dissect the cellular and molecular mechanisms, our next goal is to map the light-off jump reflex habituation neuronal circuitry and study the effect of ID genes on activity of this circuit.

Established and well-characterized *Drosophila* habituation models allow to test for genetic interactions between known and with novel candidate ID genes. Interacting genes/proteins act in common cognitive processes and can potentially be targeted by common treatment strategies. Moreover, the *Drosophila* high-throughput light-off jump reflex habituation paradigm represents a suitable cognitive assay for large-scale drug testing.

8_Frints

E3 ubiquitin ligase RLIM/*RNF12* defects lead to a novel X-linked intellectual disability disorder in which the cognitive/behavioral phenotype of carrier females is rescued by favorable nonrandom X-inactivation

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X-linked intellectual disability (XLID) is a clinically and genetically heterogeneous disorder. Still novel XLID genes are being identified. Through massively parallel sequencing of all X-chromosome exons of index males from a large cohort of 405 XLID families, likely causative protein truncating and missense variants were identified in 7 novel XLID genes (Hao Hu, et al., *Mol Psychiatry*, *in press*). One of these novel XLID genes is *RNF12/RLIM*.

We present genetic and detailed clinical results of affected men from three unrelated large XLID families carrying missense variants in *RNF12*. All amino acid changes affected highly conserved amino acid residues and co-segregated with ID in these families. Furthermore, none of the variants were present in >61,486 controls and *RNF12* does not carry loss-of-function variants (dbSNP138, ExAC Browser), which further supports pathogenicity of the variants. *RNF12* encodes the E3 ubiquitin ligase RLIM. Two of the amino acid substitutions present in the patients affect amino acids of the C-terminal zinc-finger domain of RLIM and could disturb its function. RLIM plays an important role in embryonic development by acting as a negative regulator of LIM homeodomain transcription factors through two distinct and complementary mechanisms: recruitment of the Sin3A/histone deacetylase corepressor complex and targeting the coactivator of LIM homeodomain binding proteins (LDB1/CLIM). Moreover, *RNF12* is an important regulator of the X-inactivation initiation process in mammals.

9_Kleefstra

Haploinsufficiency of MECP2-interacting transcriptional co-repressor SIN3A causes mild intellectual disability by affecting the development of cortical integrity

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The transcriptional regulatory protein swi-insensitive 3 transcription regulator family member A (SIN3A), was recently shown to enhance memory and synaptic plasticity in rodent forebrain. SIN3A interacts with MeCP2 and many other proteins in co-repressor complexes. We identified dominant mutations in *SIN3A* in patients with intellectual disability and autism spectrum disorders which prompted us to study detailed genotype-phenotype relations. As the presence of signs of cortical malformation on brain MRIs also hinted to a role of SIN3A in corticogenesis, we further studied the consequences of reduced *Sin3a* expression in mouse cortical development, by employing an *in vivo* functional knockdown essay using *in utero* electroporation. *Sin3a* downregulation led to reduced cortical neurogenesis, altered cortical layering and aberrant cortico-cortical projections, which corresponds to the observed clinical features in our patients. Overall, our data establish that haploinsufficiency of *SIN3A* is associated with a defined syndrome characterized by mild ID, a strikingly similar facial gestalt, hypermobile joints, hearing loss, and abnormal cortical development. Our studies support the role of the close MeCP2 interactor Sin3a, as a key regulator of transcriptional events important in expansion and connectivity of cortical areas.

10_Koemans**The role of EHMT1 and MLL3 in learning and memory**

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Mutations in epigenetic regulator genes are causative for neurodevelopmental disorders, such as intellectual disability (ID) and autism. Kleefstra syndrome is characterized by moderate-severe ID, autism and facial dysmorphisms due to mutations in EHMT1. It encodes the conserved Euchromatic Histone MethylTransferase 1, which mono- and dimethylates histone H3 at lysine 9. Interestingly, further mutations have been identified in other epigenetic regulator genes in patients with a phenotype that is strikingly similar to Kleefstra syndrome. One of these is MLL3 (KMT2c) involved in mono-, di- and tri-methylation of histone H3 at lysine 4. Based on these findings, we hypothesize that both proteins operate in a common epigenetic module.

Using *Drosophila* as a model, we have demonstrated that EHMT (EHMT1) and trr (MLL3) show a strong antagonistic genetic interaction, that EHMT is crucial for long-term memory formation and that abnormal H3K9me2 can be found at *Drosophila* memory genes. Our research aims to understand the downstream pathways controlled by EHMT and trr, and how these are involved in learning and memory. We performed mRNA-seq analysis of EHMT mutant and trr knockdown heads. We identified 327 up- and 796 down-regulated versus 272 up- and 341 down-regulated genes, respectively. The identified dysregulated genes in both mutants overlapped substantially (119 genes, $p=6.2 \times 10^{-23}$, hypergeometric test). We now perform ChIP-seq experiments and will present our conclusions about (common) direct and indirect target genes of EHMT and trr. However, preliminary analyses did not identify dysregulated memory genes. We hypothesize that the memory-relevant target genes might not be dysregulated at steady-state, but upon learning-induced transcriptional responses. Alternatively, relevant transcriptional changes might only occur in a subset of neurons. Therefore, the INTACT method is used to isolate genetically-labeled nuclei from the fly's memory centre, the mushroom body, before and after learning. We hope to unravel molecular pathways in neuronal and transcriptional plasticity and reveal how mutations in two different chromatin modifiers can lead to a similar human pathology.

11_Laumonnier

GABA/Glutamate synaptic pathways targeted by integrative genomic and electrophysiological explorations distinguish autism from intellectual disability

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Phenotypic and genetic heterogeneity is predominant in autism spectrum disorders (ASD), for which the molecular and pathophysiological bases are still unclear. Significant comorbidity and genetic overlap between ASD and other neurodevelopmental troubles are also well established. However little is known about the frequent observation of a wide phenotypic spectrum, even within multiplex families, associated with deleterious mutations affecting a unique gene.

We performed a clinical, neurophysiological (*in vivo* electro-encephalography - Auditory Evoked Related Potentials) and genetic (whole-exome sequencing) follow-up analysis of 2 families with known deleterious *NLGN4X* gene mutations (either truncating or overexpressing) present in individuals with ASD and/or with intellectual disability (ID). Complete phenotypic evaluation of the pedigrees showed in the ASD cases common specific autistic behavioural features and neurophysiological pattern (abnormal MisMatch Negativity in response to auditory change) that were absent in healthy parents as well as in members with isolated ID. Whole-exome sequencing identified in ASD patients from each family, a second rare inherited genetic variant, predicted deleterious and affecting either the *GRLB* or the *ANK3* genes which encode *NLGN4X* interacting proteins expressed respectively in inhibitory or in excitatory synapses. The *GRLB* and *ANK3* mutations were absent in relatives with ID as well as in controls databases.

In summary, our findings provide evidence of a double-hit genetic model focused on excitatory/inhibitory synapses in ASD, not found in isolated ID and associated with an atypical *in vivo* neurophysiological pattern linked to predictive coding.

12_Meredith

GABAergic synaptic plasticity in medial prefrontal cortex of Fmr1-KO mouse model: timing and time windows

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Imbalances in the ratio of excitatory to inhibitory signalling in the brain are common to many neurodevelopmental disorders. Significant changes in excitatory plasticity are observed in the brain of the Fmr1-KO mouse model for the developmental disorder, Fragile X syndrome. Prominent downregulation of GABAergic receptor subunits is reported in the Fmr1-KO mouse model, along with an enhanced susceptibility to epileptiform network activity and seizures. However, it is not clear how this subunit downregulation is reflected in inhibitory synaptic properties and synaptic plasticity and whether these changes occur within a specific neurodevelopmental time window.

Using patch-clamp electrophysiology, we measured GABAergic synaptic transmission and inhibitory synaptic plasticity of inputs to pyramidal neurons in layer V in the rodent medial prefrontal cortex. To characterise inhibitory development on a longitudinal axis, we recorded from juvenile pups and adolescent Fmr1-KO and wildtype littermates.

We find significantly slower kinetics of both evoked and spontaneous GABAergic synaptic currents in KO compared to wildtype mice at equivalent stages. Latency-to-onset of inhibitory synaptic responses is considerably delayed by approximately 35% in KO mice. Furthermore, at the earliest stage recorded of two weeks postnatal development, inhibitory synapses showed less short-term depression over 5, 20 and 50 Hz frequencies in KO mice.

These findings reveal evidence for changes in GABAergic signalling and plasticity at the synaptic level during early stages of brain development in the Fmr1-KO mouse model and point towards an imbalance in inhibitory regulation in the developing medial prefrontal cortex circuitry.

13_Nguyen**A miRNA signature emphasizes epigenetic misregulation in Autism Spectrum disorders**

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Autism spectrum disorder (ASD) is a neurodevelopmental disease caused by an interaction between genetic vulnerability and environmental factors. MicroRNAs (miRNAs) have emerged as key post-transcriptional regulators and are involved in multiple aspects of brain development and connectivity. Here, using olfactory mucosal stem cells biopsied from living patients, we identified a signature of four miRNAs (*miR-146a*, *miR-221*, *miR-654-5p* and *miR-656*) commonly deregulated in ASD. This signature is conserved in primary skin fibroblasts and allows discriminating between ASD and intellectual disability samples. Putative target genes of the differentially expressed miRNAs were enriched for pathways previously associated to ASD and altered levels of neuronal transcripts targeted by *miR-146a* and *miR-221* were observed in patients' cells. Further analysis of *miR-146a* revealed a substantial increase in marks of active gene transcription at the corresponding promoter. In the mouse brain, *miR-146a* displays strong expression in neuronal cells in regions important for high cognitive functions, and *miR-146a* overexpression leads to neuronal dendritic arborisation. These findings have strong diagnostic implications and emphasize the role of epigenetic deregulation in the etiology of ASD, opening new opportunities for therapeutic approaches.

14_Rajcan-Separovic**Clinical, genomic and functional characterization of 2p15.3-16.1 microdeletion syndrome**

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The 2p15-16.1 microdeletion syndrome (OMIM 612513) is a genomic disorder that we first described in two phenotypically similar individuals with intellectual disability (ID) (Rajcan-Separovic *et al.* JMG, 2007). We will present our recent work on determining the common clinical and genomic features in 23 subjects with this microdeletion (15 reported in literature and 8 new cases that we recruited) as well as efforts towards identifying the “driver” genes for this syndrome.

Clinically, all 23 cases have delayed neurocognitive development (mild to severe ID, language delay and/or ASD). Microcephaly is the most common phenotypic abnormality (20/23 subjects), while >50% of subjects have feeding problems, hypotonia, variety of head shape abnormalities (most commonly bitemporal narrowing) and consistent facial dysmorphism (telecanthus, hypertelorism, short palpebral fissures, ptosis, epicanthal folds, broad/high nasal root, smooth and long philtrum, everted lower lip, thin upper lip and palate abnormalities). Genital and digital abnormalities were also common.

Microdeletions in the 23 subjects span ~9.8 Mb (55.6 Mb – 65.4 Mb, hg19) and include 37 coding genes. The deletions are extremely variable in size, ranging from ~0.5 Kb to ~8 Mb with median deletion size of 3.14 Mb. This variability, and the existence of non-overlapping deletions within this region complicate efforts to delineate a common critical region for the 2p15-16.1 microdeletion syndrome. Instead, haploinsufficiency of several candidate genes from this region, their disturbed interaction or regulation could result in similar phenotypes. We will present the plausible candidates identified so far based on their expression in developing brain and in patient cells, frequency of deletion, haploinsufficiency scores, assessment of cellular function in patient cells, and effect on development in zebrafish knock-out model.

15_Riazuddin**GENETIC AND MOLECULAR BASIS OF INTELLECTUAL DISABILITY IN PAKISTANI POPULATION**

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Intellectual disability (ID) is a common cognitive impairment that affects 2-3% of the general population which places a huge burden on families, societies and health care system. The prevalence of ID is 2-3 fold higher in poor countries when compared with the developed world. This is attributable to poor socio-economic conditions as well as the high incidence of consanguineous marriages in some populations. It is estimated that about 20% of the human population live in communities which practice consanguineous marriages and that at least 8.5% of children have consanguineous parents. The extent of consanguinity varies among different populations in the world and is more prevalent among Muslim population. The rate of consanguineous marriages in Iran is above 40%, and in Pakistan and several Arab countries is above 50%. This provides a unique resource for studies to unravel the genetic basis of autosomal recessive ID (ARID).

In the present study we enrolled 300 families with 2-5 affected siblings from different areas of Pakistan. In our initial screening, 100 families were studied for linkage to known ID loci. Consequently, in 11 families phenotype co-segregated with known autosomal ID loci. Of the remaining families with unknown genetic causes, we selected eight families for genome wide scan using ABI STR marker panels and 100 families for whole exome sequencing (WES) of DNA samples, which revealed unique new loci for ARID in several of these families. In sixteen ARID families, mutations were found in known ID genes. These mutations include missense variations in *POMT2*, *PGAP1*, *TSHR*, *FGFR1*, *YARS* and *ALG12* genes and frameshift variation in *ZFYVE26*, *MKKS*, *APTX* and *SPG11* genes. A compound heterozygous mutation was detected in *SCN1A* gene and two canonical splice site mutations were found in *MAN2B1* and *AP4S1* gene. In one family, we found missense variants in two different X-linked genes, namely *PHF8* and *ZNF41*. Interestingly, we also identified mutations in novel ID genes in 17 ARID families. All these variations were found segregating with disease phenotype. Further support for the causality of these mutations has to come from functional studies in animal models, and for conclusive evidence, from the identification of additional mutations in these genes that are associated with concordant phenotypes.

16_Van Esch**Exome sequencing in patients with Circumferential skin creases Kunze type:
Evidence for locus heterogeneity**

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Congenital circumferential skin creases are extremely rare and children born with this feature are also referred to as 'Michelin tyre babies' based on the similarity with the mascot of the French tyre manufacturer. Some of these children have additional anomalies including typical facial dysmorphism, cleft palate, short stature and intellectual disability. For this syndrome, our group proposed the term 'Circumferential skin creases Kunze type' (Wouters *et al.*, 2011). So far, less than 10 cases have been described in literature and all occurred sporadic. In an international collaboration we collected DNA samples from 8 patients with Circumferential skin creases Kunze type.

Exome sequencing was performed on the HiSeq2000 platform for two case-parent trios as well as two additional patients with this syndrome. Data analysis revealed the presence of pathogenic mutations in either one of two interacting genes, providing evidence for genetic heterogeneity. Three additional patients with the same phenotype have also been found to carry a mutation in one of these genes. While some patients carry a heterozygous *de novo* mutation, others present with homozygous mutations. Accurate genotype-phenotype correlations are being investigated. In addition, we are performing functional analyses on protein level to elucidate the pathogenic mechanism of the mutations. This will be discussed at the meeting.

17_Yalcin**9.6% of mouse gene knockouts show abnormal neuroanatomy: a resource to identify genes related to intellectual disability in human**

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Although intellectual disability affects 1-3% of the population, it is one of the least understood health problems. It is estimated that genetic lesions account for half of the currently undiagnosed cases. Despite recent successes in identifying some of the mutations responsible, it has been suggested that up to 1,000 more genes remain to be uncovered.

The large number of intellectual disability syndromes is due to many causal pathophysiological mechanisms. The diversity of mechanisms results in an array of quantifiable neuroanatomical abnormalities. To identify genes related to intellectual disability, we are collaborating with the Sanger Mouse Genetics Project (MGP), allied to the International Mouse Phenotyping Consortium (IMPC), to systematically study the neuroanatomy of the MGP/IMPC knockout mouse strains using a standardized set of 78 brain parameters.

So far, we have assessed brain defects in 825 knockout mouse mutants. These preliminary data yielded success with the identification of 40 known intellectual disability genes including *Ap4e1*, *Cenpj*, *Chd7*, *Mcph1*, *Sc4mol* and *Ube3b* demonstrating the pertinence of our approach. We also discovered 41 other genes including *Mta1*, *Ccdc104*, *Caprin2* and *Dusp3*, which when disrupted caused modification of brain structures.

Our study is the largest screen of brain morphology from the MGP/IMPC. It shows that we can detect abnormalities in about 10% of knockout mouse mutants, and that these translate into human pathology. This offers a complementary resource to human genetic studies.

18_Zahir**Pathway analyses of whole genome sequence data identifies novel candidate Intellectual Disability genes**

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Intellectual disability (ID) is the most frequent severe life-long handicap, with a global prevalence of 1-3% (WHO). The cause remains unknown in about 30% of cases despite undergoing a plethora of clinical testing. We conducted whole genome sequencing (WGS) on a carefully selected cohort of 8 trios – patients with idiopathic ID and morphological brain defects, and both normal parents. Patient pedigrees suggested autosomal dominant inheritance.

Genomes were sequenced on Illumina HiSeq (30X). BWA, SAMtools MpileUp, Bedtools and ANNOVAR were used for re-alignment (hg19), variant calling, selection of *de novo* variants and annotation respectively. We employed a liberal filter- selecting all coding rare (MAF of <0.01 in 1000G, NHLBI-ESP and local database of 1500 genomes) heterozygous variants. We found ~30 genes with at least one hit in each patient. We conducted pathway analyses of all candidate genes for enrichment in neurodevelopment (IGA, DAVID, Panther) and refined candidate variants/genes for verification.

We independently verified *de novo* heterozygous coding SNVs in 6 genes; *SPRY4* (non-synonymous SNV), *CACNB3* (non-synonymous SNV), *SQSTM1* and *UPF1* (both in same patient), *PHF6* (stop-gain), and *ARID1B* (frame-shift). Genotype-phenotype correlations for the known ID gene *ARID1B* confirms pathogenicity. *SQSTM1* and *UPF1* each contain two adjacent *cis* mis-sense SNVs; in *SQSTM1* they cause an early termination while in *UPF1* they cause an amino-acid substitution. The variants in *PHF6*, *SQSTM1*, *CACNB3* and *SPRY4* fit accepted criteria to be likely pathogenic while we are unsure of the outcome of the *CACNB3* variant. In a subsequent validation study we found damaging variants in the coding sequence of *CACNB3*, *SPRY4*, *SQSTM1* and *UPF1* were significantly enriched in 2081 patients who presented with neurodevelopmental/neurofunctional phenotypes versus a control 2535 subjects indicating these genes are important for neurodevelopment. Further pathway analyses revealed that all 6 genes connected via a maximum two nodes to the ubiquitin proteasome degradation pathway indicating the importance of this pathway in normal brain development. Our informed approach to variant filtration has enabled the detection of novel candidate ID genes, and our data highlights an important pathway in neurodevelopment.

POSTER ABSTRACTS

1_Birling

CRISPR/Cas9 genome editing in rodents : *In vivo* and *in vitro* applications

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Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) protein 9 system has been described as a robust and multiplexable genome editing tool, enabling researchers to precisely manipulate specific genomic elements, and facilitating the elucidation of target gene function in biology and diseases.

The CRISPR/Cas9 system allows to generate insertions, deletions, duplications or substitutions at specific sites in rodents by simple pronuclear injection of the Cas9 mRNA or protein, one or more specific guide mRNA and a DNA template for specific modifications (when a specific modification is required). In many cases, this technology abolishes the need of embryonic stem cells. We have obtained deletions through non-homologous end joining (NHEJ) with efficiencies up to 70% in both mouse and rat. We are currently working on improving the use of CRISPR/Cas9 for integrating mutations by homologous recombination in order to be able to generate quicker and cost effective customized rodent models. We will apply the CRISPR/Cas9 technology to the generation of mouse model that will be phenotyped in the IMPC (<http://www.mousephenotype.org/>). We will generate KO alleles by deleting a critical exon in genes which are not currently available as targeted ES cells.

We are also using successfully the CRISPR/Cas9 system *in vitro* in ES cells to improve the targeting efficiency for projects which failed previously. We have currently recovered 5 projects for which we were not able to obtain any targeted ES cells (in some case we screened previously more than 1500 ES cells) by standard electroporation. The simple addition of a plasmid expressing the Cas9 and a guide RNA recognizing the site of insertion of the selection cassette has dramatically improved the homologous recombination rate.

A few cases of both *in vivo* and *in vitro* experiments will be presented and discussed in this poster.

2_Campagnari

Diagnostic efficiency of intellectual disability etiology by a combination of SNP arrays and targeted gene sequencing.

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Till present, half of the ID cases remain with unknown etiology after genetic and cytogenetic investigations. This work aims to evaluate the diagnostic efficient of an strategy for post-natal genome screening.

300 patients were selected from the **Center of Odonthological Assistance for Patients with Special Needs** at the University of São Paulo State, Aracatuba, Brazil. Patients presented moderate to severe intellectual disability and many of them exhibited associated dismorphic features or congenital abnormalities. Patients who had a previous diagnosis or a clear environmental cause were excluded.

Till present, 262 patients have been array genotyped (Illumina SNP Array 850K) and analysed using the BlueFuse, BlueGnome® software. We found 39.7% of patients presenting pathogenic alterations, and another 11.5% with possibly pathogenic. These alterations can be distributed in i) microdeletions associated to classic syndromes, (ii) several megabases alterations (cytogenetic resolution), iii) small alterations affecting one or few genes. In addition, 9 patients presented high fractions of their genomes in homozigosity, suggesting that consanguinity contributes to the phenotype of the patients.

Different published articles, including some from our group reported lower detection rates of pathogenic or possible pathogenic CNVs (17-23%), using array based copy number approaches.

Three factors probably contribute to this high frequency of patients with genomic unbalances found herein: **i) High Resolution of the Illumina CRC 850K platform:** higher effective resolution, considering the combination of probe density and number of probes required for making a call; **ii) Patients ascertainment:** Patients presented moderate to severe ID, frequently associated to other phenotypic alterations; **iii) Lack of previous karyotyping:** Although there is a consensus that genomic arrays should replace G-banding as first-tier cytogenetic diagnostic test for idiopatic intellectual disability or congenital abnormalities, copy number studies in developed countries had their patients already karyotyped before submitting to array screening, excluding those with cytogenetic visible alterations. Over 60% of the patients of our cohort had never been karyotyped. The patients which are negative for copy number alterations by array screening are being tested by targeted NGS for mutations in a panel of around 800 genes previously implicated in genetic disorders, at least 340 previously associated to ID. A preliminary screening of the sequencing results in 151 patients showed alterations potentially pathogenic in 64% of them.

3_Frengen

Clinical and molecular characterization of progressive encephalopathies in children

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Background. Progressive encephalopathies (PE) in children are clinically and genetically heterogeneous. They often affect brain morphology or signaling intensity on cerebral MRI, with developmental arrest and neurological regression. Metabolic defects or other mechanisms result in atrophy of existing neural cells. Spinal cord, peripheral nerves and retina may also be involved. Intellectual disability, epilepsy, ataxia, spasticity and dystonia are common. Mortality is high. Our objectives were to identify disease causing DNA variants in undiagnosed cases of PE and to perform functional analyses in novel disease genes.

Inclusion criteria. Neurological signs included: ataxia, epilepsy, dystonia, autism, spasticity syndrome, retinopathy (stationary or progressive), dementia, striatal necrosis, intellectual disability, peripheral neuropathies and encephalopathies. Cerebral MRI examinations revealed cortical atrophy, subcortical white matter changes, cerebellar degeneration, or basal ganglia abnormalities in the majority of the patients. We ruled out CNS infection, trauma, vascular accidents and sequelae after asphyxia and prematurity. Congenital anomalies were evaluated using the London Medical Database. We have collected > 70 patients in 55 families.

Methods. After karyotyping, aCGH and MLPA and/or sequencing of candidate genes, we have so far performed Whole Exome Sequencing (WES) in 55 family trios or inverted trios (two children and one parent) and continued with data filtering (population frequency, estimated severity of variants, alignment, inheritance pattern). Findings are verified with Sanger sequencing and studied *in silico*. Hypotheses about their molecular consequences are explored in *in vitro* experiments in cell lines mostly established from the patients.

Preliminary results. We have so far detected the causative gene variant in 24 of 55 families (44%). We find *de novo* mutations only in patients of Norwegian origin. Our findings are in accordance with the notion that PE can be caused by defects in a wide range of cellular functions, such neuronal signaling, energy production, peroxisomal import, GPI-anchor biosynthesis, motor neuron maintenance or riboflavin uptake. We continue Whole Genome Sequencing on selected families where we have not detected clinically relevant WES results.

4_lacono

Epigenomes of cognitive disorders

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Epigenomic programming has emerged as a fundamental process in CNS development, regulation of cellular identity and structuring of long-term information storage in postmitotic neurons. In GENCODYS (wp4), by using state-of-the-art NGS-sequencing techniques and bioinformatics analysis, we seek to disclose and dissect the epigenetic foundations of cognitive disorders (CDs) in order to achieve a better understanding of the molecular mechanisms and inspire the development of novel therapeutic approaches.

At present, we are generating epigenetic profiles of adult and developing mouse brain regions, featuring genome-wide DNA methylation, seven different histone modifications, and coding and non-coding RNA expression (as advised in the guidelines of the IEHC Reference Epigenome Standards). Exploratory datasets were generated for 11 different mutated lines, including *Ehmt1*, *Ehmt2*, *Cdkl5*, *Nr1i3*, *Med17*, *ARX*, *Dyrk1a*, *Cntnap2*, *Atp6ap2*, *Kansl1* and *17q21.31*.

Preliminary results shows that the analysis of the epigenomes allows us to pinpoint and characterize with extreme accuracy the alterations caused by the mutations, such as for instance in the *Ehmt1* +/- line, where we observe a general, localized, increase of the repressive mark H3k9me3 and a predominant repression of gene expression, which mainly targets secreted proteins (such as *semaphorin 3B*), genes responsible for cell-adhesion (with a massive dysregulation of the *protocadherin beta* cluster) and calcium ion binding genes. When looked more into the details, the set of repressed genes shows to include both well-characterized transcripts, such as *Alx4*, a transcription factor previously linked to *Potocki-Shaffer* syndrome (craniofacial anomalies and mental retardation) and enlarged parietal foramina, as well as intriguing transcripts with yet unknown roles, such as *6820431F20Rik* (which seems to be dysregulated in a *Treacher Collins* syndrome gene knockdown model).

Ongoing work includes the generation of large dataset of epigenetic profiles of several brain regions (*hippocampus*, *striatum*, *cerebellum*, *olfactory bulb* and *cortex*) at *P1*, *P7*, *P15* and *P30* developmental stages in normal and *Ehmt1* +/- mice.

By integrating the epigenomes with the phenotype data provided by the consortium members we will be able to achieve outstanding insights into the relationship between molecular pathways and phenotypes of intellectual disabilities.

5_Lopes

New insights concerning the association of *AKT3* with Microcephaly and Corpus Callosum Abnormalities in small 1q43q44 deletions: contribution of seven cases

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Microdeletions at 1q43q44 have been described as resulting in a clinically recognizable phenotype. In the last years, the report of several patients with copy number variations affecting the 1q43q44 region highlighted the *AKT3* (V-AKT MURINE THYMOMA VIRAL ONCOGENE HOMOLOG 3) gene as a likely key player in the syndrome phenotype of intellectual disability, facial dysmorphisms and microcephaly. Genotype-phenotype correlations from previous reports have implicated *AKT3* in microcephaly but not corpus callosum abnormalities. We collected seven patients with copy number variations in the 1q43q44 region: two patients with larger deletions (3.1 Mb and 3.7 Mb), four patients with smaller deletions affecting *AKT3* and *SDCCAG8* (range from 0.12 to 0.35 Mb) and one patient with a smaller quadruplication (1 Mb) affecting the entire *AKT3* gene. Both patients with larger deletions presented microcephaly, whereas the patient with a quadruplication of this region displayed the mirror phenotype: macrocephaly. Previous reports in the literature have correlated the duplication of the entire *AKT3* gene with macrocephaly but, to our knowledge, this is the first report an *AKT3* quadruplication associated with it. Among the four patients with smaller deletions, three presented a similar deletion affecting *SDCCAG8* (SEROLOGICALLY DEFINED COLON CANCER ANTIGEN 8) and the last six exons of *AKT3* and, although with overlapping deletions, they had different clinical outcomes, as one had

microcephaly while the other two had a normal occipital frontal circumference. In the fourth patient, a *de novo* deletion was found affecting the entire *AKT3* gene except for the first exon. Intriguingly, this patient presented corpus callosum hypoplasia and mild vermis hypoplasia but no microcephaly.

These cases, together with a few cases of small *AKT3* deletions inherited from asymptomatic progenitors, raise doubt concerning the straightforward relationship between *AKT3* deletion and microcephaly, confirm the existence of mirror phenotypes related to micro/macrocephaly and bring new insight to the current discussion regarding the core phenotype-associated genes within 1q43q44 deletions.

6_Meziane

Analysis of Atp6ap2 function in brain using a brain specific knock-out mouse model, Atp6ap2 Camk2a-Cre

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ATPase, H⁺ transporting, lysosomal accessory protein 2 (ATP6AP2) is an essential accessory component of the vacuolar ATPase required for lysosomal acidification and is implicated in several processes including the Wnt and MAPK signaling pathways.

Recently, ATP6ap2 mutations were reported in one family presenting intellectual disabilities with epilepsy and in one family with parkinsonism with spasticity. Surprisingly, in both families, the mutations identified give rise to the skipping of the exon 4 of the gene. In order to investigate ATP6ap2 implication in brain disorders, we generated and characterized a brain specific conditional knock-out for ATP6ap2 by crossing the ATP6ap2 floxed mouse line with the transgenic Camk2a-Cre mouse line (Mantamadiotis et al., 2002).

In the WT brain, ATP6ap2 is expressed in the cortex, hippocampus, hypothalamus, cerebellum and olfactory bulb. It is expressed in both glutamatergic and GABAergic neurons from the cortex and hippocampus. In Atp6ap2-Camk2aCre mice, we confirmed that ATP6ap2 is deleted in the Camk2a positive cells from the hippocampus and the cortex, but not in the GABAergic neurons.

The potential effects of Atp6ap2-Camk2Cre deletion were evaluated in series of behavioural tests. Atp6ap2-Camk2Cre-KO males showed increased spontaneous activity (locomotion and rears) in different situations including the openfield, the circadian activity test, and the Y-maze spontaneous alternation. They also showed increased object exploration. Evaluation of cognitive abilities showed that Atp6ap2-Camk2Cre-KO mice had altered contextual and cued memory for fear, and increased startle response. The temporally controlled mutants (Atp6ap2-camk2CreERT2) were also submitted to selected behavioural tests and showed to have comparable increased spontaneous activity and altered the fear conditioning, but at a lower extent than Atp6ap2-camk2Cre-KO mice.

We performed electrophysiological studies of the excitatory synaptic transmission at the hippocampal to basolateral amygdala projections. We analysed the implication of presynaptic ATP6ap2 in these projections after stimulation of the hippocampal projecting neurons deleted or not for ATP6ap2. We showed that the paired-pulse ratio (an estimation

of the presynaptic release probability) is increased in absence of the presynaptic ATP6ap2, pointing to a possible role of ATP6ap2 in the presynaptic compartment. Analyses of the implication of ATP6ap2 in the postsynaptic responses at the same projections are ongoing. Neuron subcellular colocalisation studies showed that ATP6ap2 is localised at the endoplasmic reticulum and at the vesicular compartment, mostly in the lysosomes. At the synapses, preliminary results showed that ATP6ap2 is mainly located in the presynaptic compartment. Additional subcellular colocalisations are ongoing with other markers of the post- and pre-synaptic compartments. Our results confirmed the role of ATP6ap2 in glutamatergic neurons, with potential implication in pre-synaptic function.

7_Moen**A protein interaction network of mental disability factors in neural stem cells.**

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Mental disabilities (MDs) such as intellectual disability (ID), autism spectrum disabilities (ASD) and schizophrenia (SZ) have a strong genetic component. Recently, many gene mutations associated with these MDs have been identified by high-throughput sequencing technology. However, it is unclear to what extent transcriptional regulators encoded by genes associated with one MD or different MDs act in the same gene regulatory pathways, which is important to appreciate the underlying etiology of an MD and the molecular relatedness of different MDs. Physical interaction between transcription factors is a strong predictor for their cooperation in gene regulation. We therefore purified MD-related transcription factors from neural stem cells, identified their interaction partners, and assembled a protein interaction network containing over 200 proteins. The network is enriched for protein factors associated with ID, ASD or SZ and enriched for protein factors encoded by evolutionary constrained genes. Our network thereby provides molecular connections between established MD factors and a discovery tool for new MD factors. We identified many interactions between protein factors associated with different MDs, and there does not appear to be clustering within the network by association with a particular MD. We give examples of interacting MD proteins cooperating in the regulation of disease-relevant genes and thereby provide molecular explanations for the gene-associated defects in patients. Our results suggest that the observed transcription factors associated with ID, ASD or SZ are part of the same transcriptional network.

8_Montjean

Characterization of Neuronal Morphology associated with Intellectual Disability and Autism Spectrum Disorders Genes

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Recent studies on the genetic causes of intellectual disability (ID) or autism spectrum disorders (ASD) let appear that numerous ID/ASD genes encode i/ proteins directly observed at synapses or ii/ proteins located in the nucleus. During brain development, memory acquisition and learning, hippocampal neurons exhibit formation of highly complex and dynamic connectivity with up to thousands synapses per neuron. Conversely, these contacts have been observed altered in several mice models of ID/ASD and in some ID or ASD situations of human pathology, suggesting a close association between ID/ASD and “synaptopathy”.

Pathology of synapses may therefore be considered as a hallmark of cognitive defects. To investigate this hypothesis, we study new ID/ASD genes described through the “Gencodys” network by systematic characterization of their ability to trigger synaptopathy in a culture dish”. In vitro differentiation of mouse primary culture of hippocampal neurons is analyzed after inactivation of each ID/ASD gene, either through in vitro RNA-interference strategy (shRNA) or through derivation of cells from knocked out mouse models. Potential defects at late stages of differentiation are evaluated by measuring neuritogenesis as well as synaptogenesis using fluorescent microscopy approaches. Correlations between neuronal phenotypes, gene function and expression pattern of each gene are searched.

This approach allow us to propose a new clustering of ID/ASD genes integrating their morphological phenotype, their expression pattern during *in vitro* differentiation and their molecular function, with the aim of proposing new knowledge-based therapeutical axis.

9_Okrouhlikova**Protein expression profiling of *Drosophila melanogaster* genes homologous to human genes implicated in cognitive disorders**

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We are creating high-resolution 3D maps of *Drosophila* protein expression during the embryonic development and in larval, pupal and especially adult brain, by which we aim to cover all stages of brain development relevant to associated pathology of studied human disease genes. We are using *Drosophila* strains that express each of the studied gene as fluorescently tagged (and thus easily trackable) protein. Tagged proteins are expressed under the control of their endogenous genomic regulatory elements and their transcription pattern should thus reflect the pattern of natural untagged gene. We hope that this atlas will significantly contribute to our understanding of their role in brain function and development.

10_Oppitz**Novel Orb2 targets involved in long-term memory in *Drosophila***

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Protein synthesis at synapses is thought to be necessary for synaptic plasticity and long-term memory. CPEB proteins, including *Drosophila* Orb2, are known to regulate local translation, potentially also in synapses. Orb2 was shown to be acutely required for long-term memory using the courtship-conditioning paradigm. To identify Orb2 mRNA targets we employed a cross-linking and immunoprecipitation approach (CLIP). We found 228 potential target genes and examined them for their role in long-term memory by pan-neuronal RNAi knockdown and subsequent courtship conditioning assay. All positive candidates were assessed for their role specifically in long-term memory by acute RNAi knockdown in adult flies and various secondary assays. In addition to genes already known to function in long-term memory we identified novel genes that are regulated by Orb2. They are mainly involved in the regulation of cytoskeletal organization, small GTPase signaling, synaptic organization and translational regulation.

11_Oudakker

Harnessing SINEUP technologies for intellectual disability disorders

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Intellectual disabilities (ID) represent a large and heterogeneous group of cognitive disorders. The genetic aetiology of ID is highly heterogeneous, with Mendelian mutations found in ~600 genes, the majority of which are very rarely mutated.

One such an ID disorder is Kleefstra syndrome (KS), which is caused by haploinsufficiency of the EHMT1 (GLP) gene, and is an example of an emerging group of ID disorders caused by genes encoding epigenetic regulators of neuronal gene activity. Core features of KS include ID, general developmental delay, childhood hypotonia, cranio-facial abnormalities and autistic-like behavioural problems. EHMT1 together with its partner EHMT2 (G9a) form a chromatin remodelling complex that catalyses euchromatic dimethylation of lysine 9 of histone H3, a histone post-translational modification that causes local compaction of histones resulting in repression of gene transcription.

Since KS patients still have one good copy of the EHMT1 gene, protein function could be restored using methods that can enhance the expression of the remaining normal copy of this gene. The novel SINEUP technology is the first example of gene-specific inducers of translation providing a versatile tool to increase protein synthesis of potentially any gene of interest. As a proof of concept we describe here the use of SINEUPs to enhance gene-specific translation of EHMT1 in cell lines.

12_Pajusalu

The diagnostic utility of single long contiguous stretches of homozygosity in patients with suspected genetic disorder

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Background: Multiple long contiguous stretches of homozygosity (LCSH) due to parental consanguinity is a well-known risk factor for autosomal recessive (AR) disorders. Although parental consanguinity is rare in Estonia, single LCSHs are often found with chromosomal microarray analysis (CMA), which is used as a first-tier diagnostic test for patients with intellectual disability and multiple congenital anomalies. Most often single LCSHs are classified as variants of unknown clinical significance. Whole exome sequencing (WES) can be now used to clarify the diagnostic utility of LCSH in patients with no parental consanguinity.

Methods: All patients who have been analysed in our centre by CMA during 2011-2014 are included in this study. HumanCytoSNP-12 array (Illumina, San Diego, CA) was used for all analyses. Patients with a single or two separate LCSHs with minimal length of 5 megabases per LCSH and total length not exceeding 27 megabases are selected for further analysis. Potential association between clinical phenotype and the genes located in LCSH regions is studied for each patient. If candidate gene(s) is found, further investigations will be performed to find a causative mutation. For this purpose WES will be preferred as it enables to investigate whether disease-causing mutations will locate to LCSH or other genomic regions.

Preliminary results: As of the date of the abstract submission we have analysed 2058 patients' CMA results. One LCSH was found in 156 (7.6%) and two in 11 (0.5%) patients. Many recurrent LCSHs were identified and excluded from further analysis. Twelve patients had a candidate gene in LCSH which causes AR disorder and could be associated with patient's phenotype. WES was performed in three of them. Disease-causing mutation was confirmed in two patients, leading to diagnoses of pyruvate kinase deficient anaemia and Marinesco-Sjögren syndrome.

Discussion: Based on these preliminary findings we suppose that if a candidate gene for patient's phenotype is located in a single LCSH it should be taken as a highly probable cause of the disorder. Nevertheless, in most cases LCHS did not encompass a good candidate gene. Further results of this ongoing study will be presented at the GENCODYS conference.

13_Philips

A founder mutation in a novel gene - A candidate gene for autosomal recessive non-syndromic intellectual disability (NSID)

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Background: Intellectual disability is a major health problem in our society. Non-syndromic intellectual disability (NSID) represents a heterogeneous group of diseases that is often a diagnostic challenge in clinical medicine.

Methods and Results: To facilitate gene identification, we applied whole-exome sequencing (WES) in a family with non-syndromic ID (NSID). The etiology of this NSID case was unknown and it originates from an isolated population in North-Eastern Finland. We found a missense variant in a novel gene on chromosome 12 in a consanguineous family with four affected males. The variant is inherited as an autosomal recessive trait. The variant is enriched in the North Eastern subisolate of Finland with a carrier frequency of 1:53, characteristic to a founder effect. The variant has a high CADD score (23.4), a high phyloP score (5.02) and was predicted to be deleterious by Condel (0.71). Another consanguineous family with two affected females and one affected male was identified from our cohort of 200 NSID patients. In addition, two heterozygous carriers with different phenotypes were also found. (this is practically the same as above: The local carrier frequency of this novel mutation was 1:42 among anonymous blood donor).

Localization studies in SH-SY5Y neuronal cells indicate that both the normal and mutated constructs are localized to the cell cytoplasm. Clinical features of the affected individuals are characterized by mild to severe NSID and delayed speech development. Our preliminary findings suggest that this novel gene may be associated with human disease.

Ongoing work: We are currently in the process of functionally characterizing this gene in drosophila.

14_Piton**Targeted molecular diagnosis of intellectual disability with or without autism: update from 217 to 275 genes and 100 to 300 patients confirms a 20-25% diagnostic efficiency and highlights genes recently identified or recurrently mutated**

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We have recently reported the results of targeted sequencing (TS) of 217 genes implicated in intellectual disability in 106 patients (mostly males) with ID and no diagnosis after CGH array and phenotype-driven testing of candidate genes (Redin et al. *J Med Genet* 2014). The diagnostic yield after stringent evaluation was 26% (23% for sporadic ID cases). We have now extended this using an improved panel of 275 genes, and have tested another 200 patients. Our current results confirm a diagnostic yield of 20 to 25%, with a higher diagnostic yield for females (35% vs 18%, *p-value=0,04*). Surprisingly, X-linked mutations were identified in males and females without any significant difference (9,8% vs 7,5%, *ns*). Our results also indicate that *de novo* mutations in few genes, such as *DYRK1A* or *TCF4*, are found recurrently in ID patients and may account each for 1-2% of cases (ie not far from the incidence of fragile X). The update of our gene list allowed us to identify mutations in confirmed ID genes, X-linked (*KIAA2022*, *PTCHD1*, *SYN1*, *CNKSR2*, etc) or autosomal (*MBD5*, *FOXP1*, *MED13L*, *CAMTA1*, *ASPM*, etc) as well as in very recently identified ID genes, such as *NAA10*, *TBR1*, *POGZ*, *TRIO* or *ZBTB20* (Primrose syndrome). We also report the first non-consanguineous and compound heterozygous case of *AP4S1* ID with spastic paraplegia.

15_Polla**Identification of novel autosomal recessive ID genes by exome sequencing in non-consanguineous families**

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The human brain is a highly complex structure and its normal development and function is critically dependent on the proper and tightly regulated activity of a large number of genes. Consequently, it is estimated that mutations in more than 2,000 genes can give rise to intellectual disability (ID). Of these genes, two thirds are still unknown. This scenario poses a major challenge for the identification of the underlying molecular causes in individual patients as one-by-one sequencing of these genes is not feasible due to scalability, speed and cost limitations. To overcome these limitations, exome sequencing can be used to identify pathogenic mutations underlying such clinically and genetically heterogenous disorders. In our study we focus on the identification of genes involved in autosomal recessive ID using exome sequencing.

We selected 106 non-consanguineous families with multiple affected family members in one generation. For 85 families, the DNA from one affected family member was analyzed by exome sequencing, while for the remaining 21 families, two affected individuals were analyzed to identify potential genetic defects. Exome sequencing data analysis of the first 61 families resulted in a total of 187 potential pathogenic variants. Sanger sequencing confirmed 61 of these, affecting 39 genes. Segregation analysis is still in progress, but so far 12 homozygous mutations in a total of 12 genes segregate with the intellectual disability within families. All of these ID candidate genes were mutated only once.

Whereas data analysis is still ongoing, the chosen approach has already shown to be a fast and accurate molecular tool for identification of potentially causal DNA variants in known and new genes involved in ARID. The causality of the confirmed potential pathogenic mutations will be established by searching for mutations in the same gene in patients with a comparable phenotype as well as by analysis of the effect of the changes on mRNA transcript and/or protein level. Depending on the physiological function of the protein, further investigations may include cell-based assays or the use of suitable model systems, such as the fruit fly or mouse.

16_Selloum

Mouse model for Schinzel-Giedion Midface Retraction Syndrome

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The Schinzel-Giedion Midface Retraction Syndrome (SGS) is characterized by severe mental retardation, facial dimorphism, and multiple congenital malformations comprising cardiac, neurological and skeletal defects. This rare autosomal dominant disease is caused by mutations in the *Setbp1* gene (SET-binding protein-1) located on chromosome 18q21.1. To date, more than 70 SGS cases have been reported.

In the context of the European consortium GENCODYS, we have generated a knock-out mouse deleted from the *Setbp1* gene using the genetically modified ES cells developed by the IKMC (*International Knock out Mouse Consortium*). Under the umbrella of the IMPC (*International Mouse Phenotyping Consortium*), we characterized this KO line, at the heterozygous stage, through a broad-based primary phenotyping pipeline covering all the essential adult organ systems and most areas of major human diseases. In addition, we submitted also the *Setbp1* mutant mice to a specific neuro-behaviour pipeline in order to explore more deeply the neurological functions.

Our mouse model of SGS displayed a number of phenotypic traits: Homozygous lethality, a major hypertrophic cardiomyopathy, hypocholesterolemia, increased fat mass, decreased spontaneous activity and susceptibility to seizures. A craniofacial analysis is currently ongoing. Many of these phenotypes can indeed be compared to the human clinical features.

17_Tastet

Creating mutations and deletions in the *Chl1-Cntn6-Cntn4* intellectual disability locus by CRISPR/Cas9 technology

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Background: CHL1, CNTN6 and CNTN4 are cell adhesion molecules of the IgCAM family. They are genomic neighbours and lost in the 3p deletion intellectual disability syndrome. They are predominantly expressed in the central nervous system and have individually also been associated with autism spectrum disorders. There is evidence that they could interact with each other or participate in the same pathways and thus regulate a same process. In order to determine the functional relationship between these Ig CAMs we took advantage of the CRISPR/Cas9 technology to create a mouse model in which the three genes are simultaneously mutated.

Methods: Guide RNAs targeting the *Chl1*, *Cntn6* and *Cntn4* genes were designed and tested in cell lines. These gRNAs were co-injected with Cas9 mRNA into mouse zygotes. The genotyping was realized from tail tissue. The regions targeted by the gRNAs were sequenced and compared to mouse ENSEMBL reference sequence. The presence of deletions between two genes was assessed by PCR. For each type of deletions, ligations of the fragments located downstream and upstream of the possible deletions were used as positive controls.

Results: We injected 400 zygotes from which 103 pups born. Sequencing revealed that two animals (#18 and #19) had mutations in all three genes. In the animal #18, the mutations observed created a stop codon and coded short Cntn6 and Cntn4 truncated proteins (41 and 26 amino acids instead of 1026 for the full length proteins). The consequences of the mutations observed in *Chl1* and on the three genes of the animal #19 are under study. Interestingly, the animal #18 also carried a deletion of 1.2 Mb between *Cntn6* and *Cntn4* genes.

Conclusion: The CRISPR/Cas9 technique successfully created two mice in which the three genes *Chl1*, *Cntn6* and *Cntn4* are simultaneously mutated. One of these carried a large deletion. This result demonstrates that this technology can be used to create deletions in the Mb range. Furthermore, these two models will be of particular interest to study the complementarity of these three cell adhesion molecules in brain development and in relation to the 3 p del intellectual disability syndrome.

18_Torres**A novel duplication in 7q33 affecting *CALD1* and *AGBL3* genes in a family with Intellectual disability (ID)**

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Background: Copy number variations (CNVs) account for a large portion of ID cases. CNVs affecting 7q33 citoband (chr7:132590000-138200000) have been described, mainly in autism studies, as well as some unbalanced translocations that disrupt genes in this region. Until now, no plausible candidate genes have been identified so far that could explain some of the phenotypes presented by patients carrying these CNVs. In this work, we describe a 7q33 duplication affecting the *CALD1* and *AGBL3* genes that co-segregates with ID.

Method: Patients with DD/ID were studied by aCGH using the Affymetrix CytoScan 750K platform (genomic coordinates are according to Human Genome Build hg19). Quantitative PCR analysis (qPCR) was performed in some cases, not only to confirm the aCGH findings but also to establish the inheritance pattern of the imbalances.

Results: We identified a family with an unreported duplication at 7q33 citoband. The proband was referred to our department at the age of 11 years owing to ID. His birth-history was uneventful and the growth parameters were normal. He was described as having mild ID (IQ=54) and an opposition behaviour. Besides strabism, he did not present any dysmorphic features. The duplication was inherited from the mother, who has both cognitive deficit and psychiatric disturbances, and was also present in his brother, similarly affected to the patient. *CALD1* and *AGBL3* genes are the only genes within this region. *CALD1* codifies for caldesmon, an actin-linked regulatory protein found in smooth muscle and non-muscle cells with numerous functions in cell motility, such as migration, invasion and proliferation, exerted via the reorganization of the actin cytoskeleton. *AGBL3* codifies for a carboxypeptidase (CCP3) that mediates tubulin deglutamylation. Recent works have shown that caldesmon plays an important role in synaptic plasticity and defects in protein-deglutamylating enzymes may be associated with neurodegeneration. Therefore, disturbance of *CALD1* and *AGBL3* genes, caused by 7q33 duplication, could underlie the phenotypes presented by these patients.

19_Tzschach**Next-generation sequencing in X-linked intellectual disability**

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X-linked intellectual disability (XLID) is a genetically heterogeneous disorder with more than 100 genes known to date. Most genes are responsible for a small proportion of patients only, which has hitherto hampered the systematic screening of large patient cohorts. We performed targeted enrichment and next-generation sequencing of 107 XLID genes in a cohort of 150 male patients. Hundred patients had sporadic intellectual disability, and 50 patients had a family history suggestive of XLID. We also analysed a sporadic female patient with severe ID and epilepsy because she had strongly skewed X-inactivation. Target enrichment and high parallel sequencing allowed a diagnostic coverage of >10 reads for ~96% of all coding bases of the XLID genes at a mean coverage of 124 reads. We found 18 pathogenic variants in 13 XLID genes (*AP1S2*, *ATRX*, *CUL4B*, *DLG3*, *IQSEC2*, *KDM5C*, *MED12*, *OPHN1*, *SLC9A6*, *SMC1A*, *UBE2A*, *UPF3B* and *ZDHHC9*) among the 150 male patients. Thirteen pathogenic variants were present in the group of 50 familial patients (26%), and 5 pathogenic variants among the 100 sporadic patients (5%). Systematic gene dosage analysis for low coverage exons detected one pathogenic hemizygous deletion. An *IQSEC2* nonsense variant was detected in the female ID patient, providing further evidence for a role of this gene in encephalopathy in females. Skewed X-inactivation was more frequently observed in mothers with pathogenic variants compared to those without known X-linked defects. The mutation rate in the cohort of sporadic patients corroborates previous estimates of 5-10% for X-chromosomal defects in male ID patients.

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